S41-011

Synthetic autotetraploid plants of *Phlox drummondii* exhibit significantly higher photosynthetic rate at C_{11} generation than the diploid progenitor.

P Vyas and I Terashima

Department of Biology, Graduate School of Science, Osaka University, 1-16, Machikaneyama-cho, Toyonaka, Osaka 560-0043, Japan.
Fax: +81 6 6850 5808 *e-mail*: pvyas@bio.sci.osaka-u.ac.jp

Key words: autotetraploid*, Phlox drummondii,* photosynthesis

Introduction

Multiplication of entire set of chromosomes or polyploidy is the most widespread process affecting evolution of higher plants. About 30% of higher plants are polyploids and, in some of the plant groups, the percent is much higher.

Polyploidization is known to affect various aspects of morphology, anatomy, biochemistry and physiology. The most immediate effect of polyploidy is observed in the changes in morphological characters, particularly in the leaf and flower size. Changes at biochemical and physiological levels are less obvious. Among the various reports some found that polyploidy increases net carbon assimilation while others showed that it decreases or does not affect photosynthesis (see Warner and Edwards, 1993). However, success of polyploids in diverse habitats suggests that it does influences physiological and biochemical aspects. Their success is often attributed to increased enzyme activities due to expression of additional genes and increased biochemical diversity due to increased heterozygosity.

In the present paper we present the effect of genome duplication on photosynthetic assimilation in artificially raised autotetraploids of *Phlox drummondii*. Changes in the photosynthetic rates were also compared among the initial and well-established latter generations of autotetraploids.

Materials and methods

Diploid and autotetraploid plants of two generations (C_0 – the generation at which the shoot of the diploid is treated with colchicine to induce polyploidy, and C_{11} – the colchicine raised autotetraploids running at 11^{th} generation) of *Phlox drummondii* were grown under controlled conditions.

Growth conditions: Seeds were sown (5-8 seeds/pot) in vermiculite in plastic pots and maintained in darkness for first 4-7 days. Once the seeds germinated the cover was removed and pots were maintained at a temperature 25/18^{o}C and 12 hours day/night regimen.

Induction of autotetraploidy: Some of the diploid seedlings at cotyledon stage were treated with colchicine solution (0.1%, w/v) for 6, 8 and 10 hours to raise the autoteraploids (C_0 generation). For colchicine treatment, seedlings were gently washed with distilled water and very small absorbent cotton swabs soaked in colchicine solution were placed in between the cotyledons where the terminal shoot bud initiates. When the seedlings reached four-leaf stage, they were transferred to individual pots. The autotetraploid nature of the plants was confirmed through chromosome counts at metaphase I and anaphase I.

Chlorophyll estimation: Chlorophyll content was measured according to Porra et al (1989).

Photosynthetic measurement: Photosynthesis was measured for autotetraploid and diploid plants using an portable gas exchange system (LI-COR 6400) at varying light intensities and constant CO_2 level (360 µmol/mol).

Leaf mass per unit area: The leaves on which photosynthetic measurements were made, were harvested and kept at 70°C for 48 hours and dry weight or leaf mass per unit area was estimated.

Results

The ploidy of the plants was confirmed by counting the chromosomes at metaphase I and anaphase I stages of meiosis. The diploid plants had 2n = 2*x* = 14 chromosomes and a few of the colchicine treated plants had 2n = 4*x* = 28 chromosomes. With the increase in the ploidy level, plants showed overall increase in the size of morphological characters, chlorophyll content, leaf mass and photosynthetic assimilation.

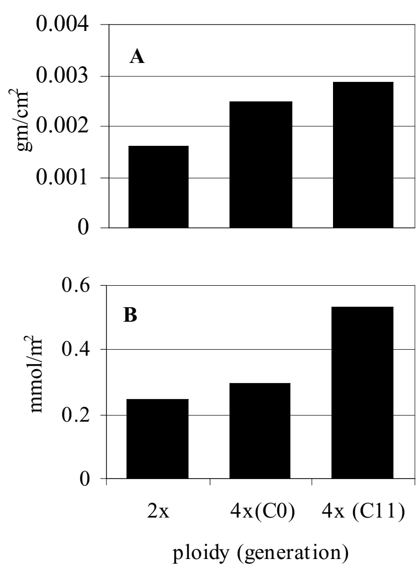

Figure 1. Leaf mass per unit area (A) and chlorophyll *a*+*b* content (B) in diploid and autotetraploids of C_0 and C_{11} generations.

However, the increase in these parameters among the tetraploids appears to be generation dependent. The chlorophyll content and leaf mass per unit area increased by 18% and 55%, respectively, in the freshly raised autotetraploid plants (C_0 generation), whereas the increase for the same was 110% and 80%, respectively, in the 11th generation (C_{11}) autotetraploid plants (Fig. 1) over the diploid progenitor.

The light saturation point of tetraploids of both the generations was much higher. The stomatal conductance was only little higher at C_0 generation while it increased about 3 times in C_{11} generation (data not shown). The photosynthetic rates were much higher in the 11th generation tetraploids than in the diploids and C_0 generation tetraploids. Net photosynthetic assimilation is increased by about 43% in C_0 generation (13 µmol CO_2 $m^{-2}s^{-1}$) and more than doubled in C_{11} generation (19 µmol CO_2 $m^{-2}s^{-1}$) (Fig. 2).

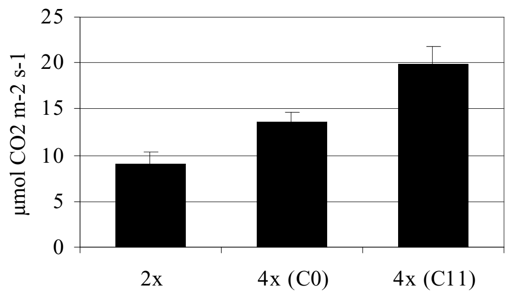

Figure 2. Maximum photosynthetic rates in diploid and autotetraploids of C_0 and C_{11} generations**.**

Discussion

Earlier, Bazzaz et al (1982) did not observe any appreciable change in the photosynthetic rates of synthetic autopolyploids of *Phlox drummondii*. However, in the present study the synthetic autotetraploids of *P. drummondii* showed significant increases in the photosynthetic activity and other related parameters. Also, the increase appears to be generation dependent. In the plants of C_0 generation the duplicated genome may be unstable or affected by other factors. However, with the progress in the generation the duplicated genome significantly affected overall performance of the plant. So far the C_0 generation tetraploid plants showed less increase over the diploid than C_{11} generation autotetraploid plants. Thus indicating that action of the duplicated genome increases with the progress of generation of the raised autotetrploid plants. It would be interesting to study whether with the further increase in the generations (beyond C_{11}) there will be further increase in photosynthetic performance or the present autotetraploids at 11th generation have already reached the limit.

Acknowledgements

The financial assistance by JSPS to PV is gratefully acknowledged.

References

Bazzaz FA, Levin DA, Levy M and Schmierbach MR (1982) *Photosynthetica* **17**: 89-92.

Porra RJ, Thompson WA and Kriedemann PE (1989) *Biochimica et Biophysica Acta* **975**: 384-394.

Warner DA and Edwards GE (1993) *Photosynthesis Research* **35**: 135-147.